Takaaki KOBARA* & Mitsuo CHIHARA*: On the taxonomy and sexual reproduction of the siphonous green alga *Pseudobryopsis hainanensis* Tseng

高原隆明*・千原光雄*: 管状緑藻ハネモモドキの分類と有性生殖について

Introduction During an excursion to the Amami Islands in March, 1978, we often collected specimens of a green alga which was apparently a member of either *Bryopsis* or *Pseudobryopsis* (=*Trichosolen*) judging by its gross morphology.

After a few days in laboratory culture the specimens matured and produced many reproductive organs near the base of the ramuli. The position of the reproductive organs is characteristic of *Pseudobryopsis* rather than *Bryopsis*. There is little information available at the present time concerning the life history of *Pseudobryopsis* and, therefore, a study of our material in laboratory culture was undertaken.

Materials and Methods The specimens were collected from the northern region of the Amami Islands at Ankyaba, on March 25 and 26, 1978, and at Sani on March 27, 1978. In both places they usually grew singly on small rocks near the low intertidal level.

The specimens were washed and brushed clean in three changes of sterilized sea water and cultured directly in 500 ml flasks stoppered with cotton plugs. The medium was aerated with an air pump and the cultures were grown at 20°C.

After a few days under these conditions many reproductive organs were formed on the plants and male and female gametes were released. Zygotes obtained were washed four times after separation from other cells utilizing their negative phototactic response. The zygotes were cultured in test tubes or on depression slides. Oyster shells and corals were also used as a substrate for germlings. The culture medium used was Schreiber's solu-

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tion and cultures were kept in an incubator under the conditions of 20°C, 14 : 10 hr (light : dark cycle) and 4000-6000 lux from 30 W cool white fluorescent lamps.

Observations and Discussion The thallus is composed of several erect axes originating from a common rhizoidal base (Fig. 1). The erect axes are mostly simple, but rarely subdichotomously branched above the middle, 1.0-4.0 cm high and 600-1000 μm wide in the lower part and 375-750 μm wide in the middle. They gradually taper towards the apex and are densely covered on all sides with thin soft ramuli that are 1.2-2.0 mm long and 40-50 μm wide in the lower swollen portion, tapering to 20-30 μm wide in the middle (Fig. 3).

Chloroplasts are discoid or lenticular and very small, measuring 2.0-4.0 μm long and 2.0-3.0 μm wide. They apparently possess no pyrenoid (Figs. 2, 9) though transparent bodies were often found within the chloro-

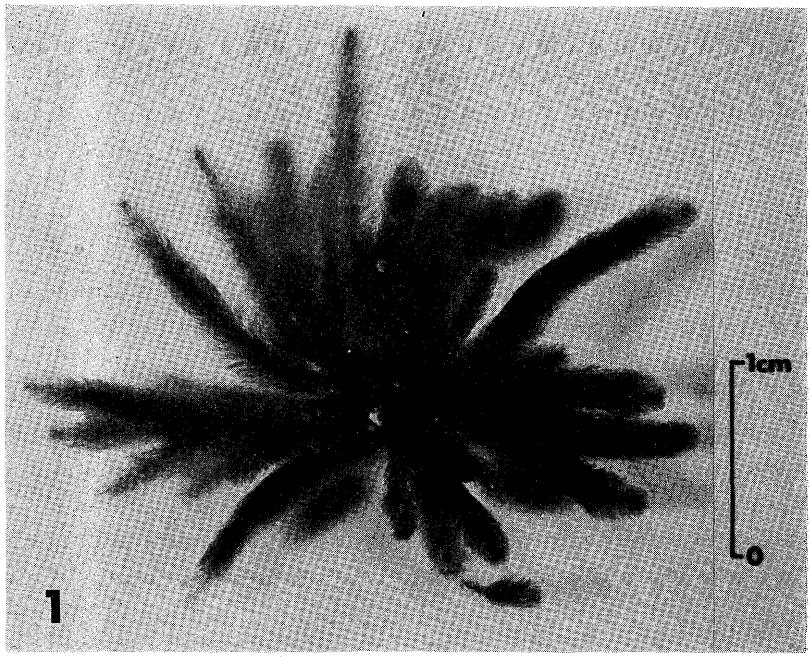
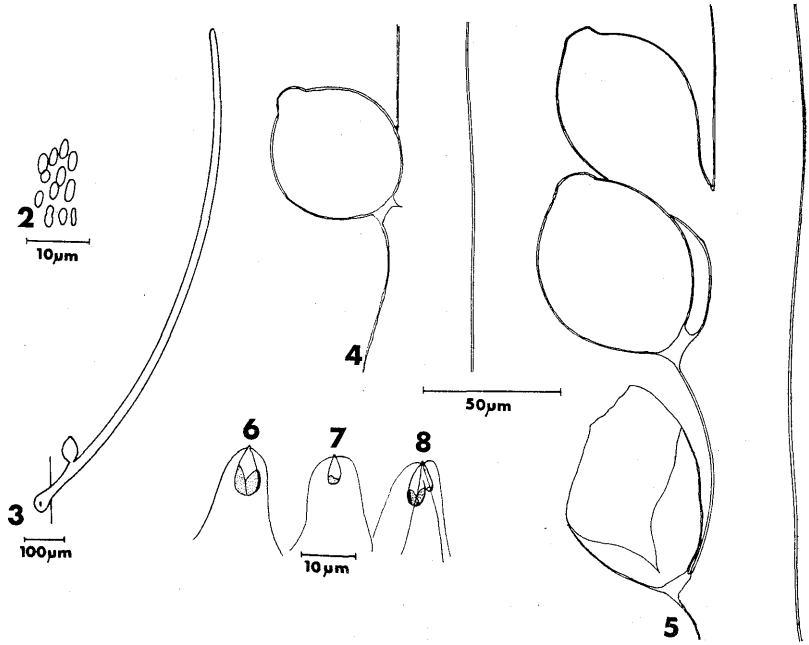


Fig. 1. Specimen of *Pseudobryopsis hainanensis* collected at Ankyaba, Amami Islands, on March 27, 1978.

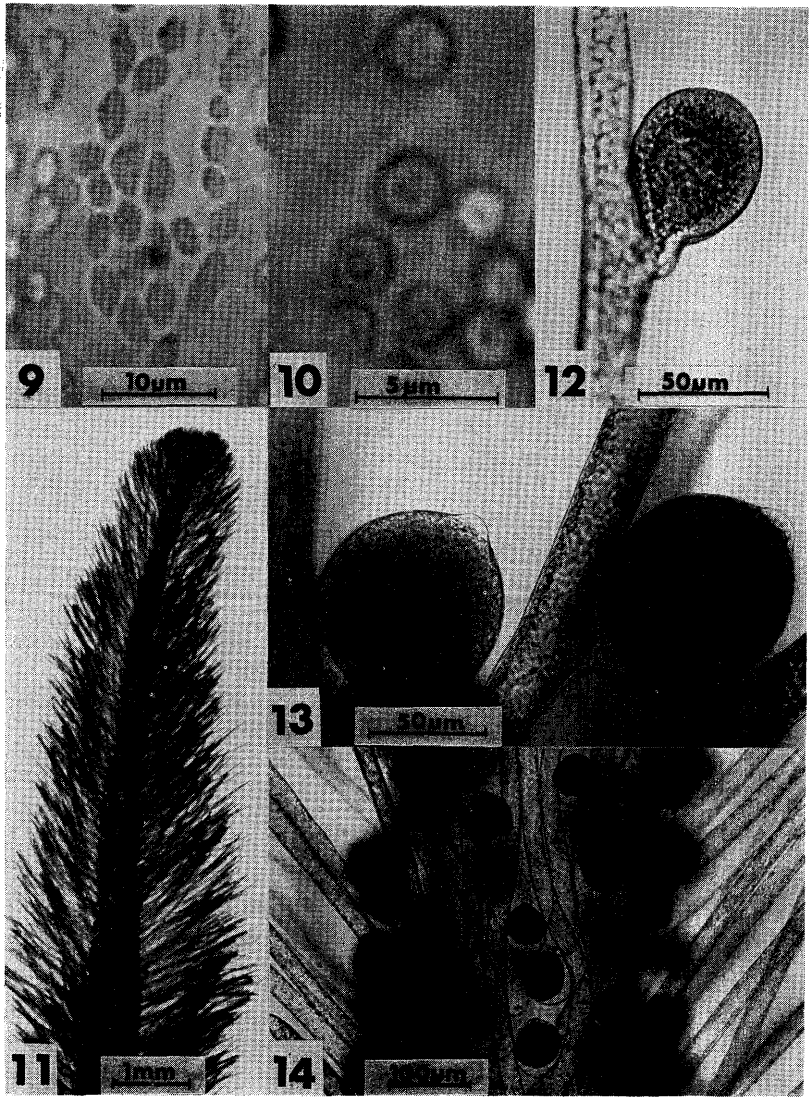


Figs. 2-8. *Pseudobryopsis hainanensis*. 2. Chloroplasts without pyrenoids. 3. A ramulus bearing a single gametangium near the main axis. 4. A matured gametangium on a ramulus. 5. A mature, immature and a ruptured gametangium on a ramulus. 6. Female gamete. 7. Male gamete. 8. Fusion of male and female gametes.

plasts, particularly in specimens preserved in formalin (Fig. 10).

Reproductive organs, which are gametangia, are borne singly or in a row of two to three per ramulus in an adaxial position on the basal part of most ramuli (Figs. 3, 4, 5, 14). Ramuli near the tip of the thalli do not have gametangia. The gametangia are broadly ovate or spherical, measuring 55-105 μm long and 35-75 μm wide. Each gametangium is furnished with a short pedicel and, when mature, with an apical papilla (Figs. 4, 13) which is not seen on young gametangium (Fig. 12).

As far as is known, two species of *Pseudobryopsis* have been reported from Japan, with their distribution restricted to the subtropical regions. The two species are *P. myura* J. Agardh[1] (Yendo, 1915) and *P. hainanensis* Tseng[1] (Ogata, 1956; Segawa & Kamura, 1960). *Pseudobryopsis oahuensis*



Figs. 9-14. *Pseudobryopsis hainanensis*. 9. Chloroplasts without pyrenoids. 10. Chloroplasts of a specimen preserved in formalin seawater, showing transparent bodies in the central portion. 11. Part of thallus bearing numerous gametangia at the basal portion. 12. A young gametangium before formation of a papilla. 13. Two matured gametangia with papillae. left; a male gametangium. right; a female gametangium. 14. Many gametangia formed adaxially at the basal part of ramuli.

Egerod[1] (Egerod, 1952; Gilbert, 1961) from Hawaii is very similar to these two species in gross morphology, size of the main axes and ramuli.

Characteristics of these three species have been compared with those of our specimens and a detailed outline is given in Table 1 and Fig. 32.

Our alga differs from *P. myura* in its smaller thallus and smaller gametangia. Likewise, its gametangia are smaller than those of *P. oahuensis*. However, it is similar to, if not identical with, *P. hainanensis* except for the reported difference in presence of pyrenoids in chloroplasts of this species.

According to Feldmann (1937, 1969) who made extensive studies on *Pseudobryopsis*, the genus can be divided into two groups on the basis of size of chloroplasts and presence or absence of pyrenoids. The first group, which includes *Pseudobryopsis myura*, the type species, has very small chloroplasts measuring 1-5 μm, without pyrenoids. The second group of species possesses larger chloroplasts, 8-12 μm long, that are provided with pyrenoids. *Pseudobryopsis hainanensis* and our specimens have the smaller chloroplasts of the first group, but there is ambiguity in reports of the presence or absence of pyrenoids.

Tseng (1936) in his description of *P. hainanensis* described the pyrenoids as follows (p. 172): "The chromatophores are roundish 2-3 μm broad. Pyrenoids are present as transparent bodies and are quite clear in the larger chromatophores." Ogata (1956) described *P. hainanensis* from Takarazima Island, located not far from our locality, and his specimen agrees well with ours but, according to him, *P. hainanensis* has pyrenoids in the chloroplasts which measure 2-3 μm.

We did not find typical pyrenoids in our material but we often found transparent bodies within the chloroplast (Figs. 9, 10) as described by Tseng (1936). We are inclined to doubt the presence of pyrenoids in chloroplasts as small as those of *P. hainanensis* and, despite the apparent discrepancy in reports of this character, we consider our specimens to be close to *P. hainanensis* Tseng. Pending examinations of the type specimen of this species, we propose the name *Pseudobryopsis hainanensis* Tseng for our alga.

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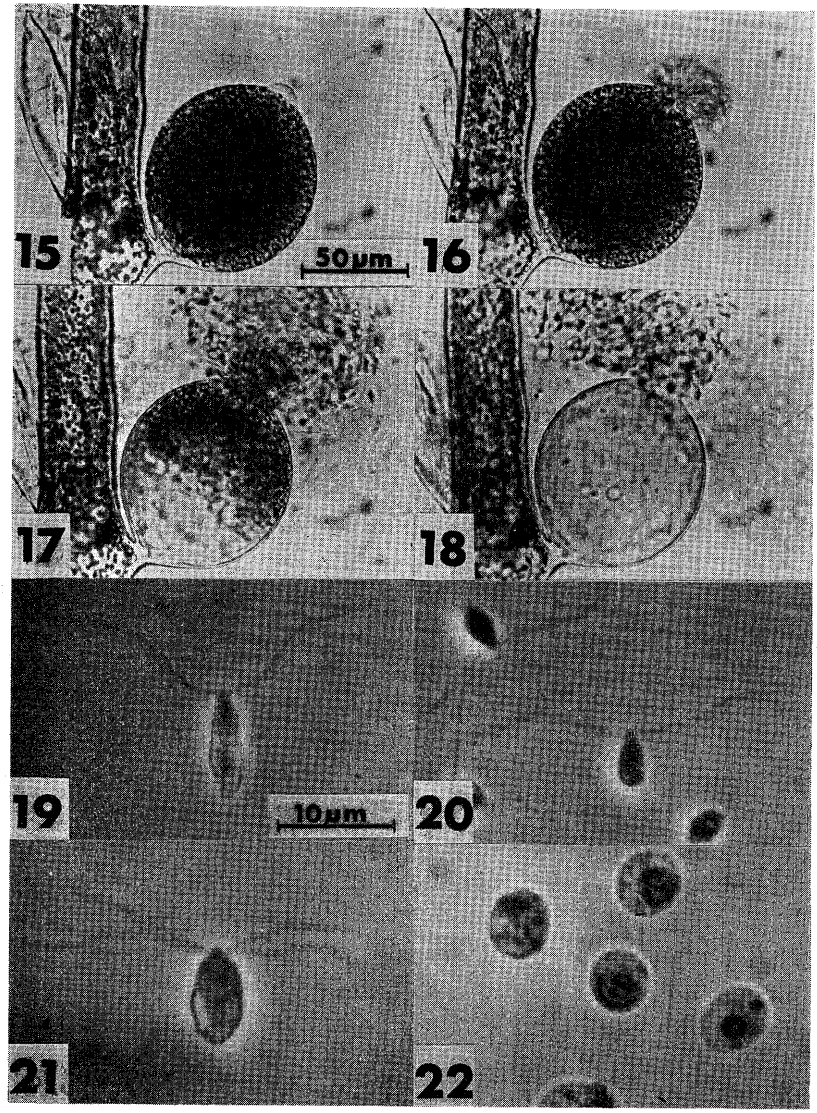
[1] *Trichosolen myura* (J. Agardh) Taylor, *T. hainanensis* (Tseng) Taylor and *T. oahuensis* (Egerod) Taylor, respectively, according to Taylor (1962).



Figs. 15-22. *Pseudobryopsis hainanensis*. 15. Gametangium immediately before the gamete release. 16. Gametangium has been broken and the release of gametes takes place. 17. Gametangium releasing gametes. 18. An empty gametangium from which the gametes have been released. 19. Female gamete. 20. Male gametes. 21. Zygote with four flagella. 22. Zygotes attached on a slide glass (after 12 hr).

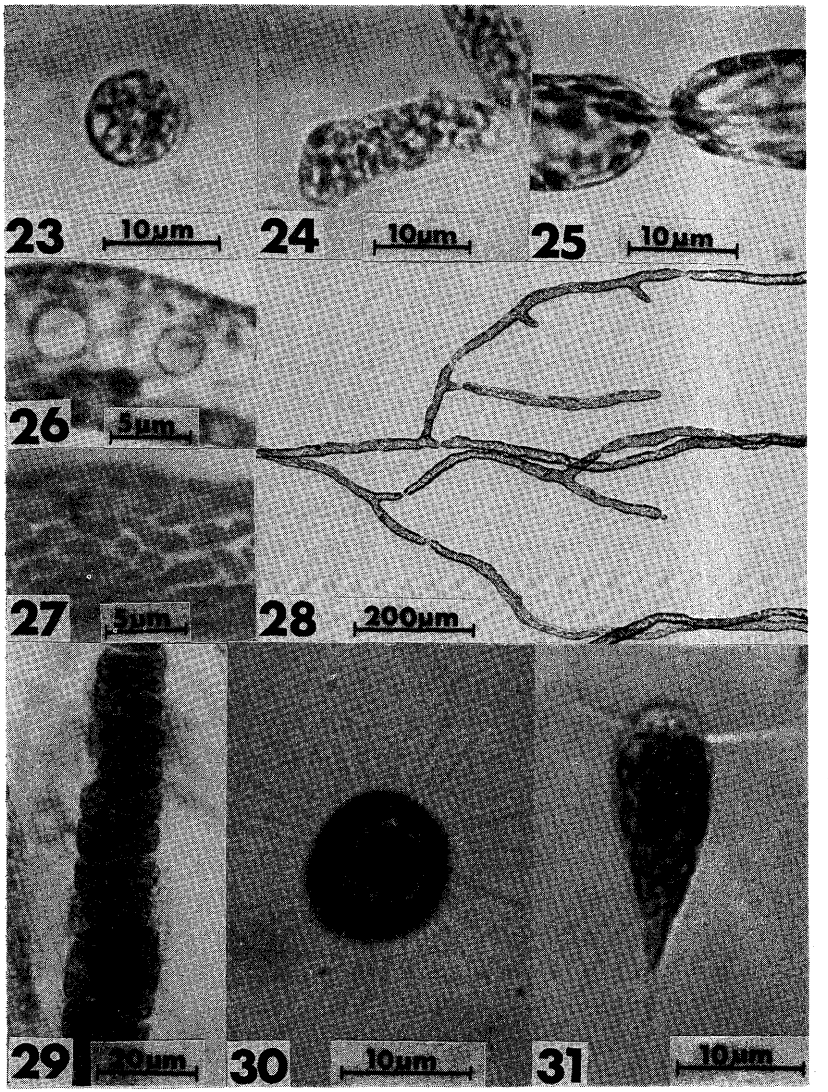
The nature of the transparent bodies in the chloroplasts and reassessment of Ogata's material is required to solve the ambiguity about putative pyrenoids in *P. hainanensis*.

Pseudobryopsis hainanensis is monoecious. Male and female gametangia occur on the same thallus and, when mature, they can be distinguished by their colors. Male gametagia become light yellowish green, whereas female gametangia become brownish orange. Gamete release occurs at the beginning of the light regime. The process of release is shown in a series of figures (Figs. 15-18). Immediately after the papilla bursts, gametes are forcibly released and, after a few minutes, the gametangium is almost empty (Figs. 16-18). Both male and female gametes are pyriform and have two equal flagella arising from the anterior end of the cell (Figs. 6, 7, 19, 20). The gametes swim vigorously. The female gametes are larger than the males, being 7-9 μm long and 4-5 μm wide. They possess two chloroplasts usually, and one eyespot (Figs. 6, 19). The male gametes are 4-5 μm long and approximately 2 μm wide. They usually contain one, or rarely two, chloroplasts, but have no eyespot (Figs. 7, 20). When these two kinds of gametes are mixed in a culture medium, copulation takes place (Figs. 8, 21). The resulting zygotes swim briefly, showing a negative phototactic response.

After becoming attached to the substrate the zygotes lose their flagella, become spheroidal (Fig. 22), increase in volume, a vacuole forms in the center of the cell, and the number of chloroplasts increases (Fig. 23). Germination of the zygote begins with the formation of a lateral germ tube (Fig. 24). The germling gives rise to a filament which later branches irregularly and sparsely (Fig. 28). The germling grows continuously, developing a long narrow prostrate filament 10-40 μm in diameter with constrictions at various places (Fig. 25). Chloroplasts in the filaments are similar to those of the plants collected in nature. They are discoid or lenticular and very small, measuring 2-4 μm long and 2-3 μm wide. They possess no pyrenoids.

Some of the filaments become erect near the distal ends as they grow densely on the surface of the substrates. However, these filaments did not develop into the erect ones similar to those found in nature. The germlings grown on shells and dead corals did not penetrate the substrates.

Information on the reproduction and life history of *Pseudobryospsis* is available in only two publications, Feldmann (1969) and Mayhoub (1974).



Figs. 23-31. *Pseudobryopsis hainanensis*. 23. Zygote with increased volume by formation of a vacuole in the center (after 48 hr). 24. A germling derived from zygote (after 5 days). 25. A constriction formed in the filament. 26. Part of filament, showing refractive granules. 27. Chloroplasts possessing no pyrenoid. 28. Portion of a filamentous germling with the constrictions in various places (after 65 days). 29. Portion of filament forming zoospores holocarpically. 30. A zoospore with many flagella lying in a transverse whorl at anterior end, viewed from anterior tip. 31. A zoospore with multiflagella and a long posterior projection, viewed from lateral.

Table 1. Comparison of size of the gametangia, main axes, ramuli and chloroplasts of three related species of *Pseudobryopsis*: *P. hainanensis*, *P. myura* and *P. oahuensis*, comprising them with the present alga. Data are based on the published papers by Tseng (1936), Ogata (1956), Feldmann (1937), Mayhoub (1974) and Egerod (1952), respectively. Up, upper portion; Lp, lower portion.

	Gametangia		Main axes		Ramuli		Chloroplasts	
	Length	Width	Height	Width	Length	Width	Size	Pyrenoids
P. hainanensis (Tseng, 1936)	52-78μm	36-52μm	2.2cm	Lp: 500μm Up: 100μm	1.2 mm	Lp: 30-36μm Up: 18-22μm	2-3μm	+
P. hainanensis (Ogata, 1956)	60-90μm	45-60μm	2.7cm (Max.)	Lp: 700μm Up: 300μm	1.2-1.8 mm	Lp: 31-39μm Up: 18-23μm	2-3μm	+
P. myura (Feldmann, 1937)	110-120μm	70-90μm	15cm (Max.)	—	—	—	3-5μm	–
P. myura (Mayhoub, 1974)	120-220μm	70-100μm	15cm (Max.)	—	—	—	—	–
P. oahuensis (Egerod, 1952)	135-150μm	60-75μm	3-5cm	400-500μm	2.5 mm	30μm	2-5μm	–
Present specimen	55-105μm	35-75μm	1.0-4.0cm	375-1000μm	1.2-2.0 mm	20-30μm	2-4μm	–

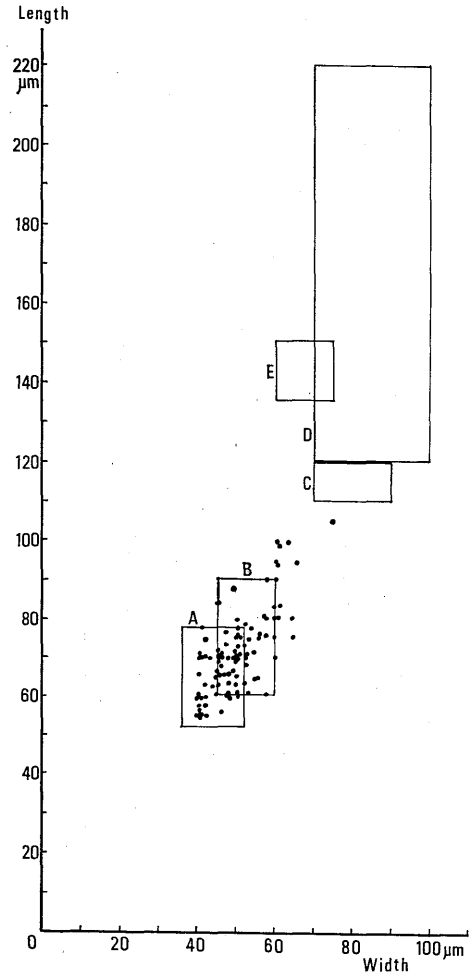


Fig. 32. Figure showing width and length of gametangia of the three species of *Pseudobryopsis* including the present alga: *P. hainanensis* (A) from Hainan Island (Tseng, 1936), *P. hainanensis* (B) from Takarazima Island (Ogata, 1956), *P. myura* (C) from Banyuls (Feldmann, 1937), *P. myura* (D) from Syria (Mayhoub, 1974), *P. oahuensis* (E) from Oahu Island (Egerod, 1952) and present alga (spots) from Amami Islands. (Data are based on the specimens collected in nature).

Both of these investigations were on *P. myura* from the Mediterranean Sea. Feldmann's study was mainly concerned with reproduction and his observations agree with ours except for one factor regarding the liberation of gametes.

Feldmann stated "Very rapidly this mass (the mass of gametes) is extruded through a hole at the apex of the gametocyst together with the central vacuole. About 10 min later the gametocyst appears empty with a mass of gametes hanging at the apex". In contrast, in our alga we observed the rapid discharge of gametes through a hole in the apical papilla. The gametes formed a jet cloud which dispersed within a few minutes (Figs. 15–18). Whether or not this difference is specific is uncertain and can only be determined afte further studies.

Mayhoub (1974) described erect axes, similar to those in nature arising directly from the fine prostrate filaments derived from the germinating zygotes in his culture of *P. myura*. He also referred to the similarities between the prostrate filaments and *Ostreobium quekettii* Bornet et Flahault. This is particularly interesting because *Ostreobium quekettii* is known as an alga capable of boring oyster shells or corals.

Five species of *Ostreobium* have been described from tropical, subtropical and temperate regions (Lukas, 1974). Of these five species our alga is closer to *O. constrictum* and *O. brabantium* in its inability to penetrate shell, and is probably closest to *O. constrictum* Lukas (1974) with which it shares characters of irregular branching, small discoid chloroplasts, and refractive granules within the cell. However, our alga is not identical with it because of the lack of the capacity to penetrate corals.

We have been able to culture erect axes from the *Ostreobium*-like filaments through the formation of stephanokont zoospores, which took place holocarpically. This result is not in agreement with that obtained by Mayhoub (1974) with *P. myura*. We will report these features in detail in the subsequent paper.

Summary A report on the structure and sexual reproduction of a recently collected green alga suggests similarities with *Pseudobryopsis hainanensis* and, in its developmental stages, with *Ostreobium*. Results of morphological studies on cultured materials are presented together with remarks on its taxonomy.

Acknowledgements We would like to thank Messrs. Isao Inoue, Jiro Tanaka and Minoru Kawaguchi, Institute of Biological Sciences, The University of Tsukuba, for their kind help in collecting the plants used in this research. Grateful thanks are also due to Dr. Richard E. Norris, University

of Washington, for his kindness in preparing the manuscript.

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奄美大島で採集したニセハネモ属 *Pseudobryopsis* (=*Trichosolen*) の1種について分類学的研究と培養による生活史の研究を行った。この藻は，葉緑体にピレノイドがない特性を除くと，Tseng (1936) が海南島から得た標本に基づいて記載した *Pseudobryopsis hainanensis* Tseng (ハネモモドキ) とよく一致する。このハネモモドキは雌雄同株，異型配偶で，接合子は発芽して *Ostreobium* の1種に似た糸状体に発達し，全実的形成によりこれに多鞭毛を冠状にもつ遊走子 (stephanokont zoospore) を生じた。この遊走子が発芽すると天然で見られるハネモモドキの体が得られる。

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